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EXAMINER
HUYNH, PHUONG N

ART UNIT	PAPER NUMBER
1644	17

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/403,882	FARINAS, JAVIER	
	Examiner	Art Unit	
	" Neon" Phuong Huynh	1644	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *Three* MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *10 January 2002* .

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-6 and 8-74* is/are pending in the application.

4a) Of the above claim(s) *19-59,61 and 62* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-6, 8-18, 60, 63-74* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other:

DETAILED ACTION

1. Claims 1-6 and 8-74 are pending.

2. Claims 19-59 and 61-62 stand withdrawn from further consideration by the examiner, 37 C.F.R. 1.142(b) as being drawn to non-elected inventions.

3. Claims 1-6, 8-18, 60 and 63-74 are being acted upon in this Office Action.

4. In view of the amendment and the substitute declaration filed by the Javier Farinas on 1/10/02, only the following objection and rejection remain.

5. The specification stands objected to because the enclosed abstract filed 1/1/02 is not on a separate sheet as required by 37 CFR 1.72(b).

6. Claims 11, 13, 14 and 15 stand rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The phrase "said cell" as recited in claims 11, 13, 14 and 15 have no antecedent basis in base claim 3. Base claim 3 recites probe or ligand conjugate.

7. The following new grounds of rejections are necessitated by the amendment filed 1/10/02.

8. The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

9. Claim 5 is rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling only for (1) a method for localizing a probe comprising a) contacting a sample comprising a cell expressing a single chain antibody that binds specifically to the ligand PhOx with a membrane permeant probe/ligand conjugate, said probe/ligand conjugate comprising i) a probe moiety such as Bodipy Fl, ii) a ligand such as PhOx that can bind with said single chain

antibody and iii) a linker moiety coupling said probe to said ligand wherein the linker is diaminopentane; (2) the said method wherein the probe is a spectroscopic probe; (3) the said method further comprises the step of detecting said probe/ligand conjugate; (4) the said method wherein the single chain antibody is membrane bound; (5) the said method wherein the single chain is comprises a fusion protein; (6) the said method wherein said detecting comprises NMR imaging, positron emission tomograph or fluorescence activated cell sorting; (7) the said method wherein the cell is eukaryotic cell, or mammalian cell; (8) the said method further comprising the steps of i) adding a stimulus to said cell and ii) detecting said probe/ligand conjugate, before and at least one time after addition of said stimulus; (9) the said method wherein said detecting comprises detecting at least one optical property of said spectroscopic probe wherein the optical property is fluorescence emission or fluorescence anisotropy; (10) a method for localizing a probe as recited in claim 60 wherein the specific binding partner is expressed from a recombinant nucleic acid consisting of SEQ ID NO: 1; (11) the said method wherein the probe provides a more intense signal when the probe/ligand conjugate is bound to the single chain antibody than when it is unbound; (12) the said method wherein the probe is a fluorescent probe that is at least about 5 fold less fluoresencent in an unbound versus bound state; (13) the said method wherein the linker is a flexible aliphatic linker or a rigid aromatic linker; (14) a method for localizing a probe comprising: a) contacting a sample comprising a cell expressing a single chain antibody that binds specifically to ligand PhOx with a probe/ligand conjugate, said probe/conjugate consisting of i) a probe moiety such as Bodipy Fl, ii) a ligand that can bind with said single chain antibody wherein the single chain binds specifically to PhOx, and iii) a non-ionic linker moiety coupling said probe with said ligand, (15) the said method wherein said probe/ligand conjugate is membrane permeant, wherein the probe provides a more intense signal when the probe/ligand conjugate is bound to said single chain antibody than when it is unbound, wherein the probe/linker conjugate consist of elements i), ii), and iii) and wherein the linker is a flexible alipahatic linker or rigid aromatic linker, **does not** reasonably provide enablement for (1) *any* method for localizing a probe comprising a) contacting a sample comprising a cell expressing *any* single chain antibody with a membrane permeant probe/ligand conjugate, said probe/ligand conjugate comprising i) a probe moiety, ii) *any* ligand that can bind with said single chain antibody and iii) a linker moiety coupling said probe to said ligand wherein said single chain antibody is *any* single chain that has at least "**30% sequence identity**" to SEQ ID NO: 1 and is capable of recognizing PhOx. The specification does not enable any person skilled in the art to

which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in **scope** with these claims.

Factors to be considered in determining whether undue experimentation is required to practice the claimed invention are summarized *In re Wands* (858 F2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988)). The factors most relevant to this rejection are the scope of the claim, the amount of direction or guidance provided, the lack of sufficient working examples, the unpredictability in the art and the amount of experimentation required to enable one of skill in the art to practice the claimed invention. The specification disclosure is insufficient to enable one skilled in the art to practice the invention as broadly claimed without an undue amount of experimentation.

The specification discloses only one single chain antibody that binds specifically to PhOx which encoded by polynucleotide "consisting" of SEQ ID NO: 1 and a specific method for localizing a probe comprising contacting a sample comprising a cell transfected with a polynucleotide consisting of SEQ ID NO: 1 that encodes a single chain antibody that binds specifically to a ligand PhOx with a membrane permeant probe Biody Fl conjugated to the ligand PhOx. The probe moiety is coupled to said ligand PhOx via a flexible aliphatic linker such as diaminopetane. The method further comprises the step of adding a stimulus to said cell and detecting said probe/ligand conjugate, before and after addition of said stimulus.

The specification does not teach how to make and use a cell expressing *any* single chain antibody that has at least "**30% sequence identity**" to SEQ ID NO: 1 and is capable of recognizing PhOx for a method for localizing a probe within the cell mentioned above. There is insufficient guidance and working examples that *any* single chain antibody with only 30% sequence identity to SEQ ID NO: 1 even bind to PhOx. A sequence with only 30% identity means 70% differences. Although the methods for determining the percent identity, such as the computer programs GCG, BLASTP, BLASTN, GeneWorks, MacVector suites NetOGlyc 2.0 and PSORT prediction, the specific conditions used by Applicant was not disclosed. The use of "percent" in conjunction with *any* of the various terms that refer to sequence identity or similarity is a problem because sequence identity between two sequences has no common meaning within the art. The term "percent" is relative and can be defined by the algorithm and parameter values set when using the algorithm used to compare the sequences. The scoring of gaps when comparing one sequence to another introduces uncertainty as to the percent of similarity between two sequences.

Skolnick *et al* teach that sequence-based methods for function prediction are inadequate and knowing a protein's structure does not tell one its function (See abstract, in particular).

Ngo *et al* teach that the amino acid positions within the polypeptide/protein that can tolerate change such as conservative substitution or no substitution, addition or deletion which are critical to maintain the protein's structure/function will require guidance (see Ngo et al., 1994, The Protein Folding Problem and Tertiary Structure Prediction, pp. 492-495).

Abaza *et al* teach even a single amino acid substitution outside of an antigenic site on protein can exert drastic efefcts on the binding specificity of the monoclonal antibody (See entire document, abstract, in particular).

Given the lack of guidance as to which specific residues within SEQ ID NO: 1 can be changed, it is unpredictable to determine which undisclosed polynucleotide sequence with only 30% identity to the full-length polynucleotide of SEQ ID NO: 1 that encodes a single chain antibody will retain its structure and function such as capable of binding specifically to PhOx. Without the specific nucleotide residues, it is unpredictable to determine which single chain antibody encoded by the undisclosed polynucleotide would be useful for transfecting the cell, in turn, the cell expressing said single chain antibody and still capable of binding to Probe/Ligand conjugate such as PhOx for the claimed method of localizing the probe. Since the specification fails to provide guidance regarding which recombinant nucleic acid resides can tolerate change, it follows that any method for localizing a probe comprising contacting a sample comprising a cell expressing a single chain antibody encoding by nucleic acid that has at least 30 % sequence to SEQ ID NO; 1 of SEQ ID NO: 1 is not enabled.

For these reasons, the specification as filed fails to enable one skill in the art to practice the invention without undue amount of experimentation. As such, further research would be required to practice the claimed invention.

10. Claims 5 and 6 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor, at the time the application was filed, had possession of the claimed invention.

The specification does not reasonably provide a **written description** of (1) *any* method for localizing a probe comprising a) contacting a sample comprising a cell expressing *any* single chain antibody with a membrane permeant probe/ligand conjugate, said probe/ligand conjugate

comprising i) a probe moiety, ii) *any* ligand that can bind with said single chain antibody and iii) a linker moiety coupling said probe to said ligand wherein said single chain antibody is *any* single chain that has at least "**30% sequence identity**" to SEQ ID NO: 1 and is capable of recognizing PhOx or *any* "**homolog**" of SEQ ID NO: 1 and is capable of recognizing PhOx.

The specification discloses only one single chain antibody that binds specifically to PhOx which encoded by a polynucleotide "consisting" of SEQ ID NO: 1 and a specific method for localizing a probe comprising contacting a sample comprising a cell transfected with a polynucleotide consisting of SEQ ID NO: 1 that encodes a single chain antibody that binds specifically to a ligand PhOx with a membrane permeant probe Biody Fl conjugated to the ligand PhOx. The probe moiety is coupled to said ligand PhOx via a flexible aliphatic linker such as diaminopetane. The method further comprises the step of adding a stimulus to said cell and detecting said probe/ligand conjugate, before and after addition of said stimulus.

Besides the specific polynucleotide of SEQ ID NO: 1 that encodes for a single chain antibody that binds specifically to PhOx for a method for localizing a probe, there is insufficient **written description** about the structure of *any* single chain antibody that has at least 30% sequence identity to SEQ ID NO: 1 and *any* single chain antibody is a homolog of SEQ ID ON: 1 which is capable of recognizing PhOx for a method for localizing a probe mentioned above. Further, Applicant discloses only one single chain antibody that binds specifically to PhOx wherein the single chain antibody is encoded by SEQ ID NO: 1. Given the lack of a written description of *any* additional representative species of single chain antibody that encoded by *any* polynucleotide encoded for a method for localizing a probe, one of skill in the art would reasonably conclude that the disclosure fails to provide a representative number of species to describe the genus. Thus, Applicant was not in possession of the claimed genus. *See University of California v. Eli Lilly and Co. 43 USPQ2d 1398.* Applicant is directed to the Revised Interim Guidelines for the Examination of Patent Applications Under the 35 U.S.C. 112, ¶ 1 "Written Description" Requirement, Federal Register, Vol. 66, No. 4, pages 1099-1111, Friday January 5, 2001.

11. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 103(a) that form the basis for the rejections under this section made in this Office Action:

A person shall be entitled to a patent unless:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

12. Claims 1-4, 6, 8, 11-14, 16-17, 60 and 63-74 are rejected under 35 U.S.C. 103(a) as being unpatentable over US Pat No. 6,017,754 (of record, Jan 2000, PTO 892) in view of Haugland *et al* (of record, Handbook of Fluorescent Probes and Research Chemicals 6th edition, 1996, pages 13-15, 18-19; PTO 892) and WO 93/11120 publication (June 1993, PTO 892).

The '754 patent teaches a method of identifying and selecting a cell to study genes of interest at a cellular level by transfecting a cell such as eukaryotic or mammalian cell CHO cell with plasmids (recombinant nucleic acid) that encode a single chain antibody (sFv) that binds specifically to phOx wherein said single chain antibody, which is a homologue of SEQ ID NO: 1 and has substantial identity to SEQ ID NO: 1 of instant application (See Fig 1A-2, Fig 6, column 1, line 54 bridging column 2 line 1, column 6, line 11, in particular). The reference cell expresses a single chain antibody (anti-phOx) which is a specific binding partner or receptor for the phOx ligand (See column 2, Summary of Invention, column 6, line 13, column 6, line 41, in particular). The reference single chain antibody comprises a fusion protein that is engineered to include coding sequence for a transmembrane domain of the human platelet derived growth factor receptor (PDGFR) or any membrane anchoring sequence so that when expressed in transfected cells, this fusion protein is anchored to the membrane via the transmembrane domain of the PDGFR so that the single chain is membrane bound (See column 10, line 4-26, in particular). The '754 patent teaches that the hapten (PhOx) as the ligand can be conjugated to a spectroscopic probe such as fluorescent (FITC) or other label via a linker moiety such as BSA to form a ligand/probe conjugate such as PhOx-BSA-FITC to allow for identification and selection of the transfected cell shortly after transfection by detecting fluorescence emission which is one of the optical properties of the FITC probe (See column 7, line 8-13, in particular). The reference FITC probe can be detect by fluorescence activated cell sorting by detecting the fluorescence emission. The said ligand (PhOx) binds to the specific binding partner such as the single chain anti-PhOx antibody non-covalently. The '754 patent further teaches that the use of a single-chained

antibody (rAB) is advantageous because the smaller size of the single chain coding sequence allows other inserted coding sequence to be longer without losing cloning efficiency since cloning efficiency is inversely related to vector size (See column 10, line 27-31, in particular).

The claimed invention as recited in claim 1 differs from the reference only by the recitation of the method for localizing a probe comprising contacting a sample with a membrane permeant probe/ligand conjugate comprising a i) probe moiety, ii) a ligand that can bind with said single chain antibody and iii) a linker moiety coupling said probe to said ligand.

The claimed invention as recited in claim 7 differs from the reference only by the recitation of the method wherein the probe/ligand conjugate is membrane permeant.

The claimed invention as recited in claims 64, 65, 68 and 72 differs from the reference only by the recitation of the method wherein the probe provides a more intense signal when the probe/ligand conjugate is bound to the single chain antibody than when it is unbound.

The claimed invention as recited in claims 66 and 69 differs from the reference only by the recitation of the method wherein fluorescent probe that is at least about 5 fold less fluorescent in an unbound versus bound state.

The claimed invention as recited in claim 67 differs from the reference only by the recitation of the method wherein the linker is a flexible aliphatic linker or a rigid aromatic linker.

The claimed invention as recited in claim 70 differs from the reference only by the recitation of the method for localizing a probe comprising contacting a sample with a probe/ligand conjugate, said probe/ligand conjugate consisting essentially of: i) probe moiety, ii) a ligand that can bind with said single chain antibody and iii) a non-linker moiety coupling said probe to said ligand.

The claimed invention as recited in claim 71 differs from the reference only by the recitation of the method wherein the probe/ligand conjugate is membrane permeant.

The claimed invention as recited in claim 73 differs from the reference only by the recitation of the method wherein the probe/linker conjugate consists of I) a probe moiety, ii) a ligand that bind with said single chain antibody, and iii) a non-ionic linker moiety coupling said probe to said ligand.

The claimed invention as recited in claims 74 differs from the reference only by the recitation of the linker is a flexible aliphatic linker or a rigid aromatic linker.

Haugland *et al* teach a membrane permeant probe such as BODIPY FL-X (See page 13-14, in particular) that contains a flexible linker such as succinimidyl esters (page 14, column 2,

first full paragraph, in particular) which is a non-ionic linker since the reference BIODPY conjugates tend to be more permeant to live cells than are conjugated of charged fluorospores (See page 14, column 2, Last full paragraph, in particular). Haugland *et al* further teach spectroscopic probes such as fluorescein, rhodamine, tetramethylrhodamine (See page 13-14, in particular). The reference BODIPY FL probe as a fluorescein substitute has excitation/emission maxima at 503 and 512 nm, respectively. Haugland *et al* teach that BODIPY conjugates tend to be more membrane permeant to live cells than are conjugates of charged fluorophores (See page 14, column 2, in particular). The reference fluorescent moiety is detected by confocal laser scanning microscopy, fluorescence microscope or flow cytometry application (fluorescence activated cell sorting) which all measure the fluorescence emission, the optical property of the probe (See page 19, in particular). Haugland *et al* further teach a method of linking the BODIPY FL dye to various protein, nucleotides (See page 15, in particular) and the probe is particularly useful for preparing conjugates of peptides, nucleotides, drugs, toxins, and other low molecular weight ligands (See page 14, column 2, second paragraph, in particular). The advantages of BODIPY FL are: (1) the spectra are insensitive to solvent polarity and pH; (2) the narrow emission bandwidth improves peak intensity over that of fluorescein; (3) little or no spectral overlap with longer wavelength dyes such as tetramethylrhodamine; and (4) greater photostability than fluorescein (See page 256, Fig 1, page 262, column 2, in particular).

WO 93/11120 publication teaches a flexible aliphatic linker such as the ε aliphatic amino group of lysine for site-selective delivery of therapeutic agent and retention thereof at the selected sites (See page 28, lines 11-32, in particular). The reference flexible aliphatic linker is lipophilic which is membrane permeant (See page 28, 12, in particular). WO 93/11120 publication teaches the N-hydroxy-succinimidyl ester derivatized linker is a particularly good reagent for reaction with lysine since the amide conjugates are very stable (See page 29, lines 4-7, in particular).

Therefore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to substitute the nonmembrane permeant linker probe conjugate such as BSA-FITC as taught by the '754 patent for the cell permeant probe linker cognate such as BIODIPY FL-X as taught by Haugland *et al* wherein the probe is coupling to the ligand via a linker moiety such as the flexible linker succinimidyl esters as taught by Haugland *et al* or the ε aliphatic amino group of lysine linker as taught by the WO 93/11120 publication for a method of detecting a probe as taught by the '754 patent, Haugland *et al* and the WO 93/11120 publication.

From the combined teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable of success in producing the claimed invention.

One having ordinary skill in the art at the time the invention was made would have been motivated to do this because Haugland *et al* teach the advantages of BODIPY FL probe are: (1) the spectra are insensitive to solvent polarity and pH; (2) the narrow emission bandwidth improves peak intensity over that of fluorescein; (3) little or no spectral overlap with longer wavelength dyes such as tetramethylrhodamine; (4) greater photostability than fluorescein (See page 256, Fig 1, page 262, column 2, in particular); and (5) useful for preparing conjugates of peptides, nucleotides, drugs, toxins, and other low molecular weight ligands for studies of cell physiology (See page 14, column 2, second paragraph, in particular). The WO 93/11120 publication teaches a flexible aliphatic linker such as the ε aliphatic amino group of lysine is useful for site-selective delivery of therapeutic agent and retention thereof at the selected sites (See page 28, lines 11-32, in particular) and the N-hydroxy-succinimidyl ester derivatized linker is a particularly good reagent for reaction with lysine since the amide conjugates are very stable (See page 29, lines 4-7, in particular). The '754 patent teaches that the use of a single-chained antibody (rAB) is advantageous because the smaller size of the single chain coding sequence allows other inserted coding sequence to be longer without losing cloning efficiency since cloning efficiency is inversely related to vector size (See column 10, line 27-31, in particular). Claim 11 is included in this rejection because the cell permeant probe such as BIODIPY FL as taught by Haugland *et al*, which can be located within the cell since it is membrane permeable. Claims 64, 65, 68 and 72 are included in this rejection because the intensity of the signal is an inherent properties of the reference probes as taught by the '754 patent, Haugland *et al* and the WO 93/11120 publication. Claims 66 and 69 are included in this rejection because the intensity of the signal such as at least about 5 fold less fluorescent in an unbound versus bound state is within the purview of one skilled in the art at the made to detect fluorescent intensity as taught by Haugland *et al.*

13. Claim 9 is rejected under 35 U.S.C. 103(a) as being unpatentable over US Pat No. 6,017,754 (of record, Jan 2000, PTO 892) in view of Haugland *et al* (of record, Handbook of Fluorescent Probes and Research Chemicals 6th edition, 1996, pages 13-15, 18-19; PTO 892) and WO 93/11120 publication (June 1993, PTO 892) as applied to claims 1-4, 6, 11-14, 16-17, 60 and 63-74 above and further in view of U. S. Pat No. 5,628,982 (of record, May 1997; PTO 892).

The combined teachings of Chestnut *et al*, Haugland *et al* and the WO 93/11120 publication have been discussed supra.

The claimed invention in claim 9 differs from the combined references only by the recitation of the method wherein the detecting is by means of NMR imaging.

The '982 patent teaches a probe such as hydroxyl-aryl metal chelates as NMR contrast agents or probes for diagnostic NMR imaging. By incorporating 2-hydroxy-aryl groups into the metal chelating ligand, the metal ion chelate NMR contrast agents (ligands) are produced which preferentially bind to specific proteins (the binding partners) in a non-covalent manner and hence the binding of the affinity of the metal chelate to the protein or distribution is enhanced (See column 1, Summary of Invention, column 15, line 38, in particular). The advantages of gadolinium ion with seven unpaired electrons are: (1) it can be used with a chelating agent having a number of open sites; (2) it can act as a contrast agent at very low dosages; (3) it can be no more toxic than iron used with a chelating agent having no open sites as taught by the '892 patent (See column 15, line 29-34, in particular).

Therefore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to substitute the Probe as taught by Haugland *et al* for the hydroxy-aryl metal chelates as NMR contrast agents for NMR imaging as taught by the '982 patent for a method of detecting a probe as taught by the '754 patent, Haugland *et al* and the WO 93/11120 publication. From the combined teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable of success in producing the claimed invention.

One having ordinary skill in the art at the time the invention was made would have been motivated to do this because the '982 patent teaches the advantages of gadolinium ion with seven unpaired electrons are: (1) it can be used with a chelating agent having a number of open sites; (2) it can act as a contrast agent at very low dosages; (3) it can be no more toxic than iron used with a chelating agent having no open sites and is a useful NMR contrast agents or probes for diagnostic NMR imaging. (See column 15, line 29-34, in particular).

14. Claim 10 is rejected under 35 U.S.C. 103(a) as being unpatentable over US Pat No. 6,017,754 (of record, Jan 2000, PTO 892) in view of Haugland *et al* (of record, Handbook of Fluorescent Probes and Research Chemicals 6th edition, 1996, pages 13-15, 18-19; PTO 892) and WO 93/11120 publication (June 1993, PTO 892) as applied to claims 1-4, 6, 11-14, 16-17, 60 and 63-74 above and further in view of U.S. Pat No. 5,324,502 (of record, June 1994; PTO 892).

The combined teachings of Chestnut *et al*, Haugland *et al* and the WO 93/11120 publication have been discussed supra.

The claimed invention in claim 10 differs from the combined references only by the recitation of the method wherein the detecting is positron emission tomography.

The '502 patent teaches a membrane permeant probe such as positron emitting gallium-68(III) cationic complex or lipophilic complex for positron emission tomography (PET). The advantage of radiopharmaceutical has the properties of prolonged retention in the targeted tissue relative to the blood levels and relative to radiopharmaceutical concentration in the adjacent non-targeted tissues (See column 1, line 61-66, in particular).

Therefore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to substitute the Probe as taught by Haugland *et al* for the positron emitting gallium-68(III) cationic complex or lipophilic complex taught by the '502 patent for positron emission tomography (PET) detection for a method of detecting a probe as taught by the '754 patent, Haugland *et al* and the WO 93/11120 publication. From the combined teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable of success in producing the claimed invention.

One having ordinary skill in the art at the time the invention was made would have been motivated to do this because the advantage of radiopharmaceutical has the properties of prolonged retention in the targeted tissue relative to the blood levels and relative to radiopharmaceutical concentration in the adjacent non-targeted tissues (See column 1, line 61-66, in particular).

15. Claim 15 is rejected under 35 U.S.C. 103(a) as being unpatentable over US Pat No. 6,017,754 (of record, Jan 2000, PTO 892) in view of Haugland *et al* (of record, Handbook of Fluorescent Probes and Research Chemicals 6th edition, 1996, pages 13-15, 18-19; PTO 892) and WO 93/11120 publication (June 1993, PTO 892) as applied to claims 1-4, 6, 11-14, 16-17, 60 and 63-74 above and further in view of Rizzuto *et al* (of record, Current Biology 5(6): 635-642, 1995; PTO 892).

The combined teachings of Chestnut *et al*, Haugland *et al* and the WO 93/11120 publication have been discussed supra.

The claimed invention in claim 15 differs from the combined references only by the recitation of the method further comprising the steps of adding a stimulus to said cell and ii) detecting said probe/ligand conjugate, before and at least one time after addition of said stimulus.

Rizzuto *et al* teach a method of localizing a probe comprising contacting a sample comprising a cell expressing a probe such as recombinant green fluorescent protein (GFP) of *Aequorea victoria* as a tool for visualizing subcellular organelles in living cells. The reference further teaches adding a stimulus (noradrenaline and histamine) to said cell and detecting the GFP conjugate before and after addition of said stimulus (See page 639-640, Use of GFP in physiological experiments, in particular) and localizing the probe (See page 637, 639, in particular). Rizzuto *et al* further teach the reporter protein can be molecular engineered to include targeting sequences which can cause them to be specifically addressed to the subcellular compartment of interest that is a useful too for monitoring cell physiology in intact living cells in response to stimulus (See abstract, in particular).

Therefore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to add a stimulus to said cell before and at least one time after addition of said stimulus as taught by Rizzuto *et al* and detecting said probe/ligand conjugate for a method of detecting a probe as taught by the '754 patent, Haugland *et al* and the WO 93/11120 publication. From the combined teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable of success in producing the claimed invention.

One having ordinary skill in the art at the time the invention was made would have been motivated to do this because Rizzuto *et al* teach the reporter protein can be molecular engineered to include targeting sequences which can cause them to be specifically addressed to the subcellular compartment of interest and is a useful too for monitoring cell physiology in intact living cells in response to stimulus (See abstract, in particular).

16. Claim 18 is rejected under 35 U.S.C. 103(a) as being unpatentable over US Pat No. 6,017,754 (of record, Jan 2000, PTO 892) in view of Haugland *et al* (of record, Handbook of Fluorescent Probes and Research Chemicals 6th edition, 1996, pages 13-15, 18-19; PTO 892) and WO 93/11120 publication (June 1993, PTO 892) as applied to claims 1-4, 6, 11-14, 16-17, 60 and 63-74 above and further in view of Youn *et al* (of record, Analytical Biochemistry 232: 24-30, 1995; PTO 892).

The combined teachings of Chestnut *et al*, Haugland *et al* and the WO 93/11120 publication have been discussed supra.

The claimed invention in claim 18 differs from the combined references only by the recitation of the method wherein the optical properties is fluorescence anisotropy.

Youn *et al.* teach a cell permeant probe such as [Ru(bpy)2(phen-ITC)]2+ for a method of detecting probe using fluorescence energy transfer immunoassay (FRET) based on the use of a ruthenium metal ligand complex. In this assay, the human serum albumin (the ligand) is covalently labeled with the donor [Ru(bpy)2(phen-ITC)]2+ and the anti-human serum albumin antibody (the receptor) is labeled with the acceptor reactive Blue 4. Upon binding of the acceptor-labeled antibody (specific binding partner) to the Ru-labeled antigen (ligand), the intensity and decay time of ruthenium metal ligand complex decrease while the anisotropy increases (See page 25, column 2, page 27, Figs 3 & 4, Table 1, in particular). One of the advantages of the Ru complex is its long decay time; since it is chemically and photochemically stable, it allows off-gating of the interfering autofluorescence and thereby increases the sensitivity of the time-resolved immunoassays. The FRET assay is more reliable because the decay times of the Ru complex are mostly independent of the overall intensity of the emission. Finally, the long decay time of the Ru complexes can be measured with simple instrumentation, and allow the measurement of rotational correlation times up to 1 μs (See page 24-25, in particular).

Therefore, it would have been obvious to one of ordinary skill in the art at the time the invention was made to substitute the Probe BIODIPY FL as taught by Haugland *et al* for the cell permeant probe such as [Ru(bpy)2(phen-ITC)]2+ which is detected by fluorescence energy transfer (florescence anisotropy) as taught by Youn *et al* for a method of detecting a probe as taught by the '754 patent, Haugland *et al* and the WO 93/11120 publication. From the combined teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable of success in producing the claimed invention.

One having ordinary skill in the art at the time the invention was made would have been motivated to do this because Youn *et al* teach the [Ru(bpy)2(phen-ITC)]2+ complex is its long decay time, chemically and photochemically stable which allows off-gating of the interfering autofluorescence and thereby increases the sensitivity of the time-resolved immunoassays (See pages 24-25 and 29, in particular). The long decay time of the Ru complexes can be measured with simple instrumentation, and allow the measurement of rotational correlation times up to 1 μs (See page 24-25, in particular).

17. No claim is allowed.

18. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

19. Any inquiry concerning this communication or earlier communications from the examiner should be directed to "Neon" Phuong Huynh whose telephone number is (703) 308-4844. The examiner can normally be reached Monday through Friday from 9:00 am to 6:00 p.m. A message may be left on the examiner's voice mail service. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Christina Chan can be reached on (703) 308-3973. Any inquiry of a general nature or relating to the status of this application should be directed to the Technology Center 1600 receptionist whose telephone number is (703) 308-0196.

20. Papers related to this application may be submitted to Technology Center 1600 by facsimile transmission. Papers should be faxed to Technology Center 1600 via the PTO Fax Center located in Crystal Mall 1. The faxing of such papers must conform to the notice published in the Official Gazette, 1096 OG 30 (November 15, 1989). The CM1 Fax Center telephone number is (703) 305-7401.

Phuong N. Huynh, Ph.D.
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April 8, 2002

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